WORLD HEALTH ORGANIZATION

ORGANISATION MONDIALE DE LA SANTÉ



STUDY GROUP ON RECOMMENDED REQUIREMENTS FOR POLIOMYELITIS VACCINE

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# SUGGESTED PROCEDURE FOR POTENCY TESTING OF POLIOMYELITIS VACCINE

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From the Division of Biologics Standards,
National Institutes of Health,
Bethesda, 14, Md, United States of America

The outline which follows presents a procedure which appears desirable to the DBS and to the Sub-committee on potency tests of the Technical Committee for potency testing of poliomyelitis vaccine using the chick.

## 1. Reference Vaccine

NIH Vaccine 1 (or other suitable preparation)

## 2. Method of Antibody Titration

The MIT method, or other equally satisfactory method, will be used for determining the presence of antibody in inoculated animals. The amount of virus in the neutralization test should be approximately 100 $TCID_{50}$. Tests using less than 32 $TCID_{50}$ or more than 1000 $TCID_{50}$ will not be considered valid.

Reference sera and type specific sera should be included in each test. Proof of the specificity of virus should be provided by the type specific serum controls. The heterotypic serum should be titrated to indicate the degree of sensitivity and reproducibility of the test system.

Two or more virus dilutions in tenfold steps may be used in the titration, if desired. If this is done, the test using the amount of virus closest to 100 $TCID_{50}$ will be used for evaluation.

## 3. Vaccine Dilutions and Serum Dilutions for Antibody Titrations

### (a) Vaccine Dilutions

Undiluted, 1/10, 1/100 for the vaccine under test. 1/4, 1/40, 1/400 for NIH Vaccine 1.

(b) Serum Dilutions

Individual sera should be run at a dilution of 1/8. Replicate tests of each serum may be performed but are not required. If replicate tests are done, all the results must be used in the calculation of antigenic extinction titres; specifically, at least 50 per cent. of cultures must be protected for the serum to be counted as positive for antibodies.

4. Number and Allocation of Chicks

A sufficient number of chicks per dilution will be used to ensure that sera from at least 30 chicks will be tested for each vaccine dilution for both the unknown and standard vaccines, which are to be tested in parallel.

The test will be done in two parts, separated by at least one week. New vaccine dilutions will be made for each part of the test and each dilution will be inoculated into at least 15 chicks. The results of the two parts of the test will be shown separately but will be combined for evaluation.

5. Chick Strain and Age of Chick

The strain of chick used should be specified in the protocol. Chicks should be of the same age; chicks seven to nine days old may be used, and should be randomly assigned for use with the test and reference vaccines.

6. Dose and Inoculation Schedule

One ml intramuscularly on days 10 and 14 with bleeding on day 21.

7. Calculations of Antigenic Extinction Titres and Potency Value

The 50 per cent. antigenic extinction end-points for the unknown and reference vaccines and for each part of the test are calculated separately by the Reed-Muench method, Karber method, or some other method, if the former methods do not apply. End-points will be computed separately for each vaccine in each part of the test.

The end-point will be computed on the basis of the proportion of positive sera at each vaccine dilution. If the reference vaccine produces less than 50 per cent. response at the initial vaccine dilution, the results cannot be used for quantitative comparison.

The result for each part of the test is calculated separately and is expressed as the ratio of the end-point dilution for the unknown to the end-point dilution for the reference vaccine. The geometric average of the two values thus computed is taken as the potency of the vaccine under test. The computation is best accomplished by computing the average (arithmetic) logarithmic end-points separately for the test and reference vaccines. The difference in average logarithms (test minus reference) is computed and the potency of the unknown is given by the anti-logarithm of this difference. A suggested potency protocol is attached.

8. Acceptable Potency Levels

A vaccine will be considered of acceptable potency if the potency ratios relative to NIH Vaccine 1, obtained and computed in accordance with the foregoing sections, are equal to or greater than 0.25 for each type.

If potency tests greater in number than specified by sections 4 and 7 are performed, results of all such tests shall be submitted to DBS and will be utilized in determining whether the vaccine is of acceptable potency.

## Monkey Potency Test Protocal

Vaccine Lot No. E-7

### Inoculation of Monkeys

| | |
|---|---|
| No. & Species | 12 Rhesus |
| Inoculation | |
| Volume | 1 cc |
| Route | I.M. |
| Dates Inoc. | 2/13, 2/20, 2/27/58 |

### Neutralization Test

| | Type I | Type II | Type III |
|---|---|---|---|
| Date of Test | | | |
| Test Number | | | |
| Virus Pool No. | | | |
| Virus Dilution | | | |
| Virus Titre | | | |
| $TCID_{50}$ | 158.5 | 251.2 | 79.4 |

### Antibody Titres

| Monkey Number | Pre-immune | | | Post-immune | | |
|---|---|---|---|---|---|---|
| | I | II | III | I | II | III |
| | None* | None* | None* | 5-5-5 | 6-7-6 | 7-7-6 |
| | None | None | None | 4-4-5 | 8-8-7 | 7-6-5 |
| | None | None | None | 5-4-4 | 7-8-7 | 5-5-5 |
| | None | None | None | 5-5-5 | 8-8-7 | 6-7-7 |
| | None | None | None | 4-5-4 | 7-8-7 | 3-4-4 |
| | None | None | None | 3-4-3 | 5-5-6 | 5-6-5 |
| | None | None | None | 4-4-4 | 8-9-9 | 8-7-7 |
| | None | None | None | 3-3-4 | 6-6-5 | 5-4-4 |
| | None | None | None | 4-4-5 | 6-7-6 | 5-7-6 |
| | None | None | None | 4-5-5 | 7-8-6 | 6-6-5 |
| | None | None | None | 6-6-7 | 7-7-8 | 6-5-6 |
| | None | None | None | 4-3-3 | 6-7-6 | 4-5-5 |

Geom. Mean Titre

| Control Serum Number | Titre Type I | Geom. Mean | Titre Type II | Geom. Mean | Titre Type III | Geom. Mean |
|---|---|---|---|---|---|---|
| II A | 6,7,7,7 | | 8,8,8,8 | | 7,6,7,7 | |
| | 7,8,7,7 | | 8,8,8,8 | | 7,7,7,6 | |
| II A 1:4 | 6,7,7,5 | 4.24 | 6,7,6,7 | 6.91 | 6,6,6,7 | 5.58 |
| | 6,6,5,5 | | 7,7,7,7 | | 7,7,6,6 | |
| II A 1:16 | 3,4,4,4 | | 4,5,5,5 | | 4,5,4,4 | |
| | 4,4,3,4 | | 5,5,5,5 | | 3,5,5,3 | |
| Ratio $\frac{\text{Test Sera}}{\text{Control Sera}}$ | 0.41 | | 1.13 | | 0.79 | |

* None designates 3 x less than 2.

## Monkey Potency Test Protocol

Vaccine Lot No. C-2

### Inoculation of Monkeys

| | |
|---|---|
| No. & Species | 12 Rhesus |
| Inoculation | |
| Volume | 1.0 ml |
| Route | I.M. |
| Dates Inoc. | 12/17/57, 12/24/57, 12/31/57 |

### Neutralization Test

| | Type I | Type II | Type III |
|---|---|---|---|
| Date of Test | | | |
| Test Number | | | |
| Virus Pool No. | | | |
| Virus Dilution | | | |
| Virus Titre | | | |
| $TCID_{50}$ | 159 | 32 | 63 |

### Antibody Titres

| Monkey Number | Pre-immune | | | Post-immune | | |
|---|---|---|---|---|---|---|
| | I | II | III | I | II | III |
| | None* | None* | None* | 6-5-5 | 7-7-7 | 6-5-5 |
| | None | None | None | 5-5-3 | 8-7-6 | 5-5-5 |
| | None | None | None | 5-4-3 | 7-7-7 | 5-4-4 |
| | None | None | None | 4-4-4 | 6-6-6 | 5-4-3 |
| | None | None | None | 3-3-2 | 7-7-7 | 3-3-3 |
| | None | None | None | 5-5-3 | 8-7-7 | 5-5-4 |
| | None | None | None | 8-7-7 | 9-9-8 | 7-7-6 |
| | None | None | None | 8-7-7 | 9-9-8 | 9-8-8 |
| | None | None | None | 7-6-6 | 9-9-9 | 7-7-7 |
| | None | None | None | 6-4-4 | 8-7-7 | 6-6-5 |
| | None | None | None | 3-3-2 | 7-6-6 | 4-4-3 |
| | None | None | None | 5-4-3 | 8-8-8 | 4-4-4 |
| | | | | | | |
| | | | | | | |
| Geom. Mean Titre | | | | 4.8 | 7.4 | 5.1 |

| Control Serum Number | Titre Type I | Geom. Mean | Titre Type II | Geom. Mean | Titre Type III | Geom. Mean |
|---|---|---|---|---|---|---|
| II A | 7,7,7,7, | | 9,8,8,8, | | 7,7,7,7, | |
| | 6,6,6,5 | | 8,8,8,7 | | 7,6,6,6 | |
| II A 1:4 | 6,6,5,5, | 5.3 | 8,7,7,7, | 6.8 | 6,6,6,6, | 5.6 |
| | 5,5,5,4 | | 7,7,6,6 | | 6,5,5,5 | |
| II A 1:16 | 5,5,5,5, | | 6,6,6,6, | | 5,5,5,5, | |
| | 4,4,4,3 | | 5,5,5,5 | | 5,4,4,3 | |
| Ratio $\frac{\text{Test Sera}}{\text{Control Sera}}$ | 0.71 | | 1.52 | | 0.71 | |

*None designates 3 x less than 2.

Sample
Chick Potency Test Protocol
Poliomyelitis Vaccine

Protocol No._____

Chick Strain_____

Vaccine Lot No _____

| | Part 1 | Part 2 |
|---|---|---|
| Volume Inoculated | _____ | _____ |
| Dates Inoculated | _____ | _____ |
| | _____ | _____ |
| Date Final Bleeding | _____ | _____ |
| Date of Serum Test | _____ | _____ |

| | Type I | | Type II | | Type III | |
|---|---|---|---|---|---|---|
| | Part 1 | Part 2 | Part 1 | Part 2 | Part 1 | Part 2 |
| Virus Pool No | _____ | _____ | _____ | _____ | _____ | _____ |
| Virus Dilution | _____ | _____ | _____ | _____ | _____ | _____ |
| Virus Titre | _____ | | _____ | _____ | _____ | _____ |
| $TCID_{50}$ | _____ | _____ | _____ | _____ | _____ | _____ |

Number of Conversions Out of Number Tested by Type

Part 1

| Vaccine Dilution | Lot (L) | | | Reference (R) | | |
|---|---|---|---|---|---|---|
| | I | II | III | I | II | III |
| 1/1 | 12/15 | 15/15 | 14/15 | | | |
| 1/4 | | | | 11/15 | 14/15 | 14/15 |
| 1/10 | 5/15 | 14/15 | 9/15 | | | |
| 1/40 | | | | 3/15 | 11/15 | 9/15 |
| 1/100 | 0/15 | 3/15 | 3/15 | | | |
| 1/400 | | | | 0/15 | 3/15 | 1/15 |
| -log 50% end-point | 0.61 | 1.59 | 1.23 | 1.03 | 1.96 | 1 70 |

Part 2

| | Lot (L) | | | Reference (R) | | |
|---|---|---|---|---|---|---|
| | I | II | III | I | II | III |
| | 13/15 | 15/15 | 12/15 | | | |
| | | | | 12/15 | 14/15 | 12/15 |
| | 7/15 | 9/15 | 9/15 | | | |
| | | | | 7/15 | 7/15 | 10/15 |
| | 1/15 | 2/15 | 2/15 | | | |
| | | | | 0/15 | 1/15 | 3/15 |
| | 0 87 | 1.27 | 1.06 | 1.37 | 1.57 | 1.77 |

| | Average End-point (L) | Average End-point (R) | Average L - Average R | Antilog (Average L-Average R) |
|---|---|---|---|---|
| Type I | 0.74 | 1.20 | -0 46 | 0.35 |
| Type II | 1 43 | 1.76 | -0.33 | 0.47 |
| Type III | 1 14 | 1.74 | -0.60 | 0.25 |

## Chick Potency Test Protocol
## Poliomyelitis Vaccine

Chick Strain _____

| | Part 1 | Part 2 |
|---|---|---|
| Volume Inoculated | 2 0 ml | |
| Dates Inoculated | 12/19/57 | 12/30/57 |
| | 1/2/58 | 1/13/58 |
| Date Final Bleeding | 1/9/58 | 1/20/58 |
| Date of Serum Test | | |

Vaccine Lot No C-2

| | Type 1 | | Type II | | Type III | |
|---|---|---|---|---|---|---|
| | Part 1 | Part 2 | Part 1 | Part 2 | Part 1 | Part 2 |
| Virus Pool No | | | | | | |
| Virus Dilution | | | | | | |
| Virus Titre | | | | | | |
| $TCID_{50}$ | 178 | 45 | 50 | 89 | 280 | 141 |

### Number of Conversions Out of Number Tested by Type

| Vaccine Dilution | Part 1 Lot (L) I | II | III | Part 1 Reference (R) I | II | III | Part 2 Lot (L) I | II | III | Part 2 Reference (R) I | II | III |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1/1 | 20/20 | 20/20 | 20/20 | | | | 19/20 | 19/20 | 19/20 | | | |
| 1/4 | | | | 13/18 | 17/18 | 17/18 | | | | 8/16 | 16/16 | 14/16 |
| 1/10 | 16/20 | 18/20 | 16/20 | | | | 11/19 | 13/19 | 14/19 | | | |
| 1/40 | | | | 9/21 | 19/21 | 15/21 | | | | 2/19 | 15/19 | 9/19 |
| 1/100 | 9/23 | 16/23 | 15/23 | | | | 4/19 | 8/19 | 5/19 | | | |
| 1/400 | | | | 2/22 | 3/22 | 3/22 | | | | 1/20 | 3/20 | 0/20 |
| -log 50% end-point | 1.69 | 2.09 | 1.95 | 1.33 | 2.07 | 1.88 | 1.23 | 1.55 | 1.44 | 0.75 | 1.03 | 1.44 |

| | Average End-point (L) | Average End-point (R) | L - R | Antilog L - R |
|---|---|---|---|---|
| Type I | 1.46 | 1:04 | +0.42 | 2:63 |
| Type II | 1.82 | 1:55 | +0.27 | 1.82 |
| Type III | 1.69 | 1.66 | +0.03 | 1.07 |

# Chick Potency Test Protocol
## Poliomyelitis Vaccine

Chick Strain _____

| | Part I | Part 2 |
|---|---|---|
| Volume Inoculated | 1.0 cc IM | 1.0 cc IM |
| Dates Inoculated | 3/20/58 | 3/28/58 |
| | 4/3/58 | 4/11/58 |
| Date Final Bleeding | 4/10/58 | 4/18/58 |
| Date of Serum Test | _____ | _____ |

Vaccine Lot No. E-7

| | Type I | | Type II | | Type III | |
|---|---|---|---|---|---|---|
| | Part 1 | Part 2 | Part 1 | Part 2 | Part 1 | Part 2 |
| Virus Pool No. | ____ | ____ | ____ | ____ | ____ | ____ |
| Virus Dilution | ____ | ____ | ____ | ____ | ____ | ____ |
| Virus Titre | ____ | ____ | ____ | ____ | ____ | ____ |
| $TCID_{50}$ | 501.2 | 158.5 | 63.1 | 78.4 | 89.1 | 158.5 |

Number of Conversions Out of Number Tested by Type

| Vaccine Dilution | Part 1 Lot (L) I | Part 1 Lot (L) II | Part 1 Lot (L) III | Part 1 Reference (R) I | Part 1 Reference (R) II | Part 1 Reference (R) III | Part 2 Lot (L) I | Part 2 Lot (L) II | Part 2 Lot (L) III | Part 2 Reference (R) I | Part 2 Reference (R) II | Part 2 Reference (R) III |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1/1 | 17/18 | 18/18 | 18/18 | | | | 17/18 | 18/18 | 18/18 | | | |
| 1/4 | | | | 16/18 | 18/18 | 18/18 | | | | 14/18 | 18/18 | 16/18 |
| 1/10 | 11/18 | 18/18 | 15/16 | | | | 6/18 | 16/18 | 15/18 | | | |
| 1/40 | | | | 2/17 | 14/17 | 14/17 | | | | 7/17 | 15/18 | 14/18 |
| 1/100 | 0/16 | 6/16 | 7/16 | | | | 2/18 | 8/18 | 7/18 | | | |
| 1/400 | | | | 2/17 | 6/17 | 4/17 | | | | 2/18 | 7/18 | 5/18 |
| -log 50% end-point | 1.13 | 1.80 | 2.06 | 1.10 | 2.21 | 2.15 | 0.85 | 1.81 | 1.70 | 1.37 | 2.29 | 2.07 |

| | Average End-point (L) | Average End-point (R) | L - R | Antilog L - R |
|---|---|---|---|---|
| Type I | 0.90 | 1.23 | -0.24 | 0.57 |
| Type II | 1.80 | 2.25 | - .45 | 0.35 |
| Type III | 1.88 | 2.11 | - .23 | 0.59 |